

Title: Measurements to support translation of liquid biopsies into routine clinical practice for personalised cancer management

Abstract

Early detection and personalised management strategies can substantially increase the survival of cancer patients. A new approach, which enables the multi-parametric analysis of the biomarkers (e.g. cells, nucleic acids and proteins) that are shed into the blood by tumours, needs to be developed to provide earlier diagnosis, personalised therapies, “real-time” disease monitoring and relapse detection. However, trace level detection is challenging and there are few standards. A metrology framework is therefore needed to bring these precision medicine approaches into routine clinical practice, to ensure compliance with EU regulations, and to improve sensitivity, comparability and data analysis for accurate and reliable results.

Keywords

Liquid biopsy, personalised/precision medicine, non-communicable diseases, cancer, multi-parametric measurements, multivariate data analysis, molecular/cell diagnosis, protein biomarkers, circulating tumour cells, circulating tumour nucleic acid

Background to the Metrological Challenges

Cancer accounts for a quarter of all EU deaths. Earlier detection coupled with tailored treatment strategies has been shown to substantially increase survival. Therefore, precision medicine approaches with personalised treatments need to be developed. A new less invasive approach involves using liquid biopsies to collect the cell, gene and protein biomarkers that are shed from solid tumours into the blood or other body fluids. When coupled with advanced cell and ‘omics based measurements, using technologies such as Next Generation Sequencing, digital PCR, flow cytometry, bioimaging, mass cytometry and spectrometry, this approach could provide a step-change in cancer treatment by allowing disease progress and treatment responses to be monitored in “real-time”. There is also the potential for “universal” disease screening as the following three types of biomarkers can be analysed: i) circulating tumour cells (CTCs) via cell surface associated protein or receptor complexes or intracellular targets e.g. nucleic acids and proteins, ii) cell-free circulating tumour biomarkers such as DNA, mRNA, microRNAs and proteins, and iii) circulating microvesicles e.g. exosomes.

Diagnostic devices, including genetic oncology tests and companion diagnostics (i.e. liquid biopsies), need to comply with the In Vitro Diagnostic Regulation (Regulation (EU) 2017/746) and this includes a requirement to demonstrate the metrological traceability of calibrator and control material values. However, this recent requirement has led to validation issues and a need to increase the supply of reference materials so that traceability and compliance can be demonstrated. Similarly, the requirement for clinical laboratories to seek accreditation to ISO 15189 (Medical laboratories — Particular requirements for quality and competence) has driven a growing need for external quality assessment and for supporting reference measurement systems. Several liquid biopsy based assays have passed these regulatory hurdles and have entered clinical use including a test for EGFR-mutations in patients with non-small cell lung cancer and the OncoBEAM®RAS CRC test for patients with metastatic colorectal cancer.

The detection and quantification of biomarkers is technically challenging due to the low concentrations present in blood. Pre-analytical aspects, such as sample storage and extraction, are also critically important and currently suffer from a lack of best practice guidance. These issues were further highlighted by a recent external quality assessment scheme which found that current ctDNA detection approaches lack harmonisation and generate variable results. Therefore, test materials need to be prepared to address this and to determine the main uncertainty sources. Advanced measurement procedures also need to be developed and validated for the detection and quantification of circulating tumour cells (CTCs) from 1 CTC per mL to 10 CTC per mL of

blood. Similarly, reference measurement procedures using advanced molecular approaches need to be developed and validated to support the detection and quantification of the circulating tumour nucleic acid biomarkers from 0.0001 ng to 5 ng per mL of blood. In addition, measurement procedures are needed for the detection of tumour derived circulating biomarkers such as protein, microRNA and epigenetic (DNA methylation) markers.

Multidisciplinary approaches that combine existing nucleic acid, cell and protein liquid biopsy tests need to be developed and there is also a need for measurement comparability. Therefore, multi-parametric data analysis methods such as PCA, machine learning classifiers, logistic regression, neural networks and support vector machines need to be established for assessing the reliability, sensitivity and uncertainty of the multiparametric biomarker panels and to ensure appropriate data quality for wider translation to routine clinical use.

These metrology approaches need to be developed to support the translation of liquid biopsies into routine clinical practice for personalised cancer management. This approach needs to address the inherent technical challenges associated with detecting trace levels of biomarkers and the current lack of guidelines, standards, reference methodologies and quality control materials.

Objectives

Proposers should address the objectives stated below, which are based on the PRT submissions. Proposers may identify amendments to the objectives or choose to address a subset of them in order to maximise the overall impact, or address budgetary or scientific / technical constraints, but the reasons for this should be clearly stated in the protocol.

The JRP shall focus on the development of the underpinning metrology to improve the sensitivity, specificity, accuracy, traceability and comparability of liquid biopsy assays for translation into routine clinical practice for improved personalised cancer diagnosis and management.

The specific objectives are

1. To develop and validate measurement procedures for the detection and quantification of circulating tumour cells (CTCs) from 1 CTC per mL to 10 CTC per mL of blood.
2. To develop and validate reference measurement procedures using molecular approaches to support the detection and quantification of the circulating tumour nucleic acid biomarkers from 0.0001 ng to 5 ng per mL of blood, and measurement procedures for the detection of tumour derived circulating biomarkers such as protein, microRNA and epigenetic (DNA methylation) markers. Objectives 1 and 2 should include the development of test materials for external quality assurance and laboratory comparison studies to determine the main uncertainty sources.
3. To develop multidisciplinary approaches that combine existing nucleic acid, cell and protein liquid biopsy tests, and to establish multi-parametric data analysis methods for assessing the reliability, sensitivity and uncertainty of multi-parametric biomarker panels.
4. To support the translation of the metrology approaches for liquid biopsies into the clinic. This should include the dissemination of the measurement and assessment methods that will improve the accuracy, reliability and comparability of results in pre-clinical and clinical trials and routine practice. This process should include the establishment of a European network, including NMIs and stakeholders, to support training and standardisation relating to precision medicine, particularly to liquid biopsies.
5. To facilitate the take up of the technology and measurement infrastructure developed in the project by the measurement supply chain (accredited laboratories, instrumentation manufacturers), standards developing organisations (NAWG/CAWG, ISO/TC 212, EN/TC 140, ISO REMCO WG3) and end users (hospitals and health centres).

These objectives will require large-scale approaches that are beyond the capabilities of single National Metrology Institutes and Designated Institutes, and it is expected that multidisciplinary teams will be required. To enhance the impact of the research, the involvement of the appropriate user community such as medical practitioners, hospitals and industry is strongly recommended, both prior to and during methodology development.

Proposers should establish the current state of the art, and explain how their proposed project goes beyond this.

EURAMET expects the average EU Contribution for the selected JRPs in this TP to be 1.8 M€, and has defined an upper limit of 2.1 M€ for this project.

EURAMET also expects the EU Contribution to the external funded partners to not exceed 35 % of the total EU Contribution across all selected projects in this TP.

Potential Impact

Proposals must demonstrate adequate and appropriate participation/links to the “end user” community, describing how the project partners will engage with relevant communities during the project to facilitate knowledge transfer and accelerate the uptake of project outputs. Evidence of support from the “end user” community (e.g. letters of support) is also encouraged.

You should detail how your JRP results are going to:

- Address the SRT objectives and deliver solutions to the documented needs,
- Feed into the development of urgent documentary standards through appropriate standards bodies,
- Transfer knowledge to the health sector.

You should detail other impacts of your proposed JRP as specified in the document “Guide 4: Writing Joint Research Projects (JRPs)”

You should also detail how your approach to realising the objectives will further the aim of EMPIR to develop a coherent approach at the European level in the field of metrology and include the best available contributions from across the metrology community. Specifically, the opportunities for:

- improvement of the efficiency of use of available resources to better meet metrological needs and to assure the traceability of national standards
- the metrology capacity of EURAMET Member States whose metrology programmes are at an early stage of development to be increased
- organisations other than NMIs and DIs to be involved in the work

Time-scale

The project should be of up to 3 years duration.